PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference DEX-0027	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/03170	International filing date *(day/month/year)* 12 FEBRUARY 1999	Priority date *(day/month/year)* 23 FEBRUARY 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant DIADEXUS LLC		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before the Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14 SEPTEMBER 1999	05 MAY 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer NANCY A. JOHNSON for
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/03170

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-17 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

[X] the claims:
pages 18-20 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of

[X] the drawings:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages none

[X] the claims, Nos. none

[X] the drawings, sheets/~~fig~~ none

5. [X] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-12	YES
	Claims	13-15	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-15	NO
Industrial Applicability (IA)	Claims	1-15	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 13-15 lack novelty under PCT Article 33(2) as being anticipated by any of WO 98/05349 or Yamashita (Clin. Chim. Acta 1994). Both references disclose a method of monitoring progression, remission, response to therapy and stabilization of prostate, breast, ovarian or testicular cancer in a patient comprising measuring PLA_2 levels in a biological fluid sample, which is the same as that claimed. WO 98/05349 discloses such a method for diagnosing and monitoring the progression or remission of prostate cancer (see p.2, lines 18-21). Yamashita (Clin. Chim. Acta 1994) discloses such a method for monitoring cancerous tumors, including breast tumors (see p. 92 and 95) and notes that "M-PLA_2 levels decreased and even returned to normal levels after tumor resection," were tumor resection is interpreted to be the same as the claim limitation a "therapy of the cancer" (p. 97).

Claims 1-12 lack an inventive step under PCT Article 33(3) as being obvious over Yamashita (Clin. Chim. Acta 1994). The teachings of Yamashita, of a method of monitoring progression, remission, response to therapy and stabilization of prostate, breast, ovarian or testicular cancer in a patient comprising measuring PLA_2 levels in a biological fluid sample, have been discussed in the above paragraph. Yamashita does not teach monitoring a single patient to determine the onset of metastasis (claims 1-9) or monitoring ovarian or testicular cancer in a patient. However all such methods are prima facie obvious over the teachings of Yamashita. Yamashita teaches that PLA_2 levels are higher in patients "with distant metastasis" (see Figure 2C) compared to patients without metastasis. Therefore, it would be obvious to monitor a single patient for increases in PLAs, for the onset of metastasis (claims 1-9), in view of the teachings of Yamashita, that PLA_2 levels increase with metastasis. While Yamashita does not teach the detection of ovarian or testicular cancer (claims 10-12), such methods would be prima facie obvious in view of the teachings of Yamashita, that PLA_2 levels are elevated in a wide range of cancers (see "Blood Samples," p. 92).

Claims 1-15 have industrial applicability as defined by PCT (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): G01N 33/53, 53/567; C07K 16/00 and US Cl.: 435/7.2, 7.21, 7.23, 7.91; 7.92; 436 546; 530/387.1

I. BASIS OF REPORT:

5. (Some) amendments are considered to go beyond the disclosure as filed:
NONE

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
Article 33(4).

Claims 1-12 meet the criteria set out PCT Article 33(2).
---------- NEW CITATIONS ----------
NONE

TENT COOPERATION TRE[illegible]Y

From the INTERNATIONAL BUREAU

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
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in its capacity as elected Office

Date of mailing (day/month/year) 25 October 1999 (25.10.99)	
International application No. PCT/US99/03170	**Applicant's or agent's file reference** DEX-0027
International filing date (day/month/year) 12 February 1999 (12.02.99)	**Priority date** (day/month/year) 23 February 1998 (23.02.98)
Applicant BURCZAK, John et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

14 September 1999 (14.09.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer Maria Kirchner Telephone No.: (41-22) 338.83.38

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: METHODS USING PLA_2 AS A MARKER OF METASTASES AND FOR THE DIAGNOSIS OF SELECTED CANCERS

(57) Abstract

The present invention provides methods of monitoring a cancer in a patient which has not metastasized for the onset of metastasis by measuring PLA_2 levels in the patient. Also provided are methods for diagnosing a cancer in a patient and monitoring progression, remission, response to therapy and stabilization of a cancer in a patient based upon PLA_2 levels.

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METHODS USING PLA2 AS A MARKER OF METASTASES AND FOR THE DIAGNOSIS OF SELECTED CANCERS

INTRODUCTION

This application is a continuation of application Serial No. 09/175,504, filed October 20, 1998 which is a continuation-in-part of application Serial No. 09/111,938, filed July 8, 1998, pending, which is a continuation-in-part of provisional application Serial No. 60/075,504, filed February 23, 1998.

FIELD OF THE INVENTION

This invention relates to use of PLA_2 as a metastatic marker for monitoring cancers which have not metastasized for the onset of metastasis and for diagnosing metastatic cancers. PLA_2 is demonstrated herein to be a metastatic marker for a number of different metastatic cancers including prostate, breast, colorectal, ovarian and testicular cancer. This invention also relates to methods of monitoring selected cancers including breast, ovarian and testicular cancer for progression, remission, response to therapy and stabilization in patients by monitoring PLA_2 levels in these patients. Further, the invention relates to methods which aide in identification of cancers, including testicular and ovarian cancer, among individuals who have not yet been diagnosed with these cancers by detection of elevated PLA_2 levels.

BACKGROUND OF THE INVENTION

Extracellular Phospholipase A_2 (PLA_2) enzymes appear to mediate a variety of responses including cellular proliferation, chemotaxis and inflammation. There are two major groups of extracellular PLA_2 enzymes: pancreatic or group I; and rheumatoid arthritis synovial fluid (RASF) or group II. The group I enzymes function in digestion and in modulating proliferation and chemotaxis. Currently, RASF-PLA_2 is predominantly believed to play a role in inflammatory responses including arthritis, septic shock and lung injury. The level of RASF-PLA_2 is regulated at the mRNA level by a variety of agents including interleukin-6, interleukin-1 and tumor necrosis factor, all of which are involved in inflammatory responses. As used hereinbelow "PLA_2" refers to group II or RASF-PLA_2. PLA_2 includes native PLA_2 (whole or a breakdown product), a native complex of molecules including PLA_2, or native chemically modified PLA_2.

Membrane-associated phospholipase A_2, M-PLA_2, has been disclosed as one of five factors suspected to play a role in the tumor cell processes and metastasis in human breast cancer (Yamashita et al., *Surgery*, 1995, 117, 601-608). Further, higher M-PLA_2 levels have been reported in breast cancer patients with distant metastasis and in patients with scirrhous carcinoma as compared to other histological types (Yamashita, *Cancer*, 1994, 69, 1166-1170). PLA_2 has also been reported to be expressed exclusively in gastric cancer cells with a low grade of differentiation and appears to be intensified in the invading zone of the tumor (Murata et al., *Br. J. Cancer*, 1993, 68(1), 103-111). Elevated M-PLA_2 was also detected in three gastric cancer cell lines (MKN28, KATO III and AZ521), in a pancreatic cancer cell line (SUIT-2), in a colonic cancer cell line (SW1116) and a hepatoblastoma cancer cell line (HuH-6). (Yamashita et al., *Clinica Chimica Acta*, 1994, 228, 91-99). In addition, patients with T2-T4 tumors or stage II-IV cancers of the lung, breast and

digestive organs had significantly higher levels of M-PLA_2 in comparison to stage I and T1 cancers.

PLA_2 levels have also been reported to be increased in peritoneal and pleural effusions in patients with various types of cancers including gastric, breast, pancreatic, bile duct, lung, liver, esophageal and uterine cancer and cirrhotic ascites (Abe et al., *Int. J. Cancer (Pred. Oncol.)*, 1997, 74 245-250).

In addition, U.S. Patent 5,747,264 discloses a diagnostic assay for detection of PLA_2 protein or PLA_2 mRNA in cells, tissues or body fluids which can be used to detect the presence of cancers, and in particular, prostate cancer. This method of quantifying PLA_2 protein levels is particularly useful for discriminating benign prostatic hyperplasia and prostate cancer.

It has now been found that levels of PLA_2 in a patient can be used to diagnose and monitor various cancers for the onset of metastasis. It has also been found that determining the amount of PLA_2 in a bodily fluid of a patient is useful in diagnosing various cancers and in monitoring progression, remission, response to therapy and stabilization of certain cancers in patients.

SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the present invention to provide a method of monitoring various cancers which have not metastasized for the onset of metastasis which comprises identifying a patient having a cancer that is not known to have metastasized and measuring an amount of PLA_2 in a biological fluid obtained from the patient, wherein elevated levels of PLA_2 in the sample are indicative of metastasis. As demonstrated herein, PLA_2 is a metastatic marker for cancers, including prostate, breast, colorectal, ovarian and testicular cancer.

It is another object of the present invention to provide a method of diagnosing a metastatic cancer in a patient which comprises obtaining a sample of biological fluid from a patient; and detecting the level of PLA_2 in a sample of biological fluid, wherein elevated levels of PLA_2 in the sample are indicative of metastatic cancers.

It is another object of the present invention to provide a method for monitoring cancers, including breast, ovarian and testicular cancer, for progression, remission, response to therapy and stabilization in patients suffering from such cancers which comprises measuring levels of PLA_2 in biological fluid samples obtained from the patient at selected times, wherein an increase in the measured levels of PLA_2 over time is indicative of progressive cancer, a decrease in the measured levels of PLA_2 over time is indicative of remission or response to therapy of the cancer, and no change in the measured levels of PLA_2 over time is indicative of stabilization of the cancer.

It is another object of the present invention to provide a method to aide in identification of ovarian or testicular cancer, among individuals who have not yet been diagnosed with such cancers which comprises detecting elevated PLA_2 levels in a biological fluid obtained from a patient suspected of suffering from cancer.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

DESCRIPTION OF THE INVENTION

Procedures used for detecting, diagnosing, monitoring, staging, and prognosticating cancer are of critical importance to the outcome of patients suffering from cancer. Cancer patients are closely monitored following initial therapy and during adjuvant therapy to determine response to therapy and to detect persistent or recurrent disease or metastasis. Another important step in managing cancer is to determine the stage of the patient's disease. Stage determination has potential prognostic value and provides criteria for designing optimal therapy. Generally, pathological staging of cancer is preferable over clinical staging because the former gives a more accurate prognosis.. However, clinical staging would be preferred were it at least as accurate as pathological staging because it does not depend on an invasive procedure to obtain tissue for pathological evaluation. Staging of cancer would be improved by detecting new markers in cells, tissues or bodily fluids which could differentiate between different stages of invasion. Accordingly, there is a need for cancer markers which are more sensitive and specific in detecting cancer recurrence and for increasingly sensitive and accurate methods for the staging of a cancer in humans to determine whether or not such cancer has metastasized and for monitoring the progress of a cancer in a human which has not metastasized for the onset of metastasis.

For example, cancer of the prostate is the most prevalent malignancy in adult males, excluding skin cancer, and is an increasingly prevalent health problem in the United States. In 1996 in the United States alone, it was estimated that 41,400 deaths would result from this disease, indicating that prostate cancer is second only to lung cancer as the most common cause of death in the same population. If diagnosed and treated early, when the cancer is still confined to the prostate, however, the chances of cure are significantly

higher. Further, treatment decisions for an individual are linked to the stage of prostate cancer present in that individual. A common classification of the spread of prostate cancer has been established by the American Urological Association (AUA). The AUA divides prostate tumors into four stages, A to D. Stage A, microscopic cancer within prostate, is further subdivided into stages A1 and A2. Sub-stage A1 is a well-differentiated cancer confined to one site within the prostate. Treatment generally includes observation, radical prostatectomy, or radiation. Sub-stage A2 is a moderately to poorly differentiated cancer at multiple sites within the prostate. Treatment generally includes radical prostatectomy or radiation. Stage B, which is characterized by a palpable lump within the prostate, is also subdivided into stages B1 and B2. In sub-stage B1 the cancer forms a small nodule in one lobe of the prostate. In sub-stage B2 the cancer forms large or multiple nodules, or occurs in both lobes of the prostate. Treatment for both sub-stages B1 and B2 involves either radical prostatectomy or radiation. Stage C is a large cancer mass involving most or all of the prostate and is also further subdivided into two stages. Sub-stage C1 is characterized by the cancer forming a continuous mass that may extend beyond the prostate. Sub-stage C2 is characterized by the cancer forming a continuous mass that invades the surrounding tissue. Treatment for both sub-stages C1 and C2 is radiation with or without drugs to address the cancer. The fourth stage, Stage D is metastatic cancer and is also subdivided into two stages. Sub-stage D1 is characterized by the cancer appearing in the lymph nodes of the pelvis. Sub-stage D2 is characterized by the cancer appearing in tissues beyond the lymph nodes. Treatment for both sub-stages D1 and D2 is systemic drugs to address the cancer as well as pain.

However, current prostate cancer staging methods are limited. Existing methods for diagnosing prostate cancer such as prostatic specific antigen (PSA), digital examination, and

transurethral ultrasound tests have difficulty discriminating between prostate cancer stages A, B, C, and D. In fact, the existing PSA diagnostic tests detect 20-28% of patients with benign prostatic hyperplasia (BPH) and 62-81% of prostate cancer patients with PSA blood levels above approximately 99% of the normal population. In addition, as many as 50% of prostate cancers initially staged as A2, B, or C have metastasized and are actually stage D. However, accurate identification of metastasis is important because patients with metastatic cancers have a poorer prognosis and require significantly different therapy than those with localized cancers.

An incorrect initial diagnosis also occurs in up to 25% of patients with testicular tumors. However, the lifetime probability of developing testicular cancer is 0.2% for an American white male. The cause of testicular cancer is unknown, however, it is associated with both congenital and acquired factors. From a treatment standpoint, testicular cancer is divided into two major categories, nonseminomas and seminomas. In the commonly used staging system for nonseminomas, a stage A lesion is confined to the testis; in stage B there is regional lymph node involvement in the retroperitoneum; and in stage C, there is distant metastasis. For seminomas, a stage I lesion is confined to the testis; in stage II, the lesion has spread to the retroperitoneal lymph nodes; and in stage III, the lesion has supradiaphragmatic nodal or visceral involvement.

The most common symptom of testicular cancer is painless enlargement of the testis. Acute testicular pain resulting from intertesticular hemorrhage occurs in about 10% of cases. Patients are often asymptomatic upon presentation but about 10% may exhibit back pain, cough or lower extremity edema. A testicular mass or diffuse enlargement of the testis can be detected by physical examination in most cases. Several biochemical markers are used for the diagnosis and

treatment of this disease including human chorionic gonadotropin (hCG), alpha-fetoprotein and LDH. However, as evidenced by the high rate of initial misdiagnosis, better markers are required.

The most widely used biomarker for epithelial ovarian cancer, CA125, has also been reported to lack sensitivity and specificity (Xu et al. *JAMA*, 1998, 280 (8), 719-723). In 1995, an estimated 26,600 new cases of ovarian cancer were diagnosed in the United States. Approximately one in 70 women will develop ovarian cancer during her lifetime. An estimated 14,500 deaths in 1995 resulted from ovarian cancer. It causes more deaths than any other cancer of the female reproductive system.

Ovarian cancer often does not cause any noticeable symptoms. Some possible warning signals, however, are an enlarged abdomen due to an accumulation of fluid or vague digestive disturbances (discomfort, gas or distention) in women over 40; rarely there will be abnormal vaginal bleeding. Periodic, complete pelvic examinations are important; a Pap test does not detect ovarian cancer. Annual pelvic exams are recommended for women over 40.

As with most cancers, the risk of ovarian cancer increases with age. The rate is highest among women over 60 years of age. Women who have never had children are twice as likely to develop ovarian cancer as women who have. Women who already have been diagnosed with breast, intestinal or rectal cancers appear to be at increased risk of developing ovarian cancer. Early age of first pregnancy, early menopause and the use of oral contraceptives appear to reduce the risk of ovarian cancer.

Surgical treatment usually involves the removal of one or both ovaries, the uterus and the fallopian tubes. If the cancer is detected early, especially in younger women, it is possible that only the cancerous ovary will be removed. Radiation and chemotherapy options also are available to

prevent or slow the recurrence of the ovarian cancer or the spread of cancer to other parts of the body following surgery.

It has now been found that PLA_2 levels are significantly higher in human patients with metastatic cancer as compared to cancer which has not yet metastasized.

For example, it has now been found that PLA_2 is significantly higher in human patients with metastatic Stage D prostate cancer as compared to prostate cancer which has not yet metastasized, i.e cancer in Stage A, B, or C. PLA_2 levels have also been demonstrated to be elevated in patients suffering from other progressive metastatic cancers such as breast cancer, colorectal cancer, testicular cancer and ovarian cancer. Accordingly, the present invention relates to methods of diagnosing metastatic cancer and monitoring cancers which have not metastasized for the onset of metastasis.

In this method, a patient with cancer not known to have metastasized is identified. This is accomplished by a variety of means known to those of skill in the art. For example, in the case of prostate cancer, patients are typically diagnosed with prostate cancer following digital rectal examination, serum levels of prostate specific antigen (PSA), transrectal ultrasound, and/or needle biopsy of the prostate and surrounding tissue. For ovarian cancer, patients are typically diagnosed with ovarian cancer following pelvic examination. CA125 is the most widely biomarker for detection and management of epithelial ovarian cancer. Recently, plasma lysophosphatidic acid (LPA) levels have been suggested as a potential biomarker for ovarian cancer and other gynecological cancers (Xu et al. *JAMA,* 1998, 280 (8), 719-723). In the case of testicular cancer, patients are typically diagnosed with testicular cancer following physical examination or detection of biochemical markers including human chorionic gonadotropin (hCG), alpha-fetoprotein and LDH. A sample of bodily fluid is then obtained from this patient. Bodily fluids useful in

the present invention include blood, urine, saliva, or any other bodily secretion or derivative thereof. By blood it is meant to include whole blood, plasma, serum, or any derivative of blood, preferably serum. PLA_2 protein or mRNA levels in the sample of bodily fluid are then determined.

Assay techniques that can be used to measure PLA_2 protein or mRNA encoding PLA_2 in a bodily fluid sample derived from a patient having cancer are well-known to those of skill in the art. Such assay methods include, without limitation, radioimmunoassays, reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry assays, *in situ* hybridization assays, competitive-binding assays, Western Blot analyses, ELISA assays and proteomic approaches: two-dimensional gel electrophoresis (2D electrophoresis) and non-gel based approaches such as mass spectrometry or protein interaction profiling. Among these, enzyme immunoassays, and in particular enzyme linked immunosorbent assays (ELISAs) are frequently preferred to detect a gene's expressed protein in biological fluids. An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to PLA_2, preferably a monoclonal antibody. In addition, a reporter antibody generally is prepared which binds specifically to PLA_2. The reporter antibody is attached to a detectable reagent such as a radioactive, fluorescent or enzymatic marker, for example horseradish peroxidase enzyme or alkaline phosphatase. To carry out the ELISA, antibody specific to PLA_2 is incubated on a solid support, *e.g.*, a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin (BSA). Next, the bodily fluid sample to be analyzed is incubated in the dish, during which time PLA_2 binds to the specific antibody attached to the polystyrene dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to PLA_2 and linked to a

detectable reagent, *i.e.* alkaline phosphatase, is placed in the dish resulting in binding of the reporter antibody to the bound PLA_2. Unattached reporter antibody is then washed out and bound reporter antibody is detected. For example, if the detectable reagent is alkaline phosphatase, reagents for alkaline phosphatase activity, including a colorimetric substrate are added to the dish. Immobilized alkaline phosphatase, linked to PLA_2 antibodies, produces a colored reaction product. The amount of color developed in a given time period is proportional to the amount of PLA_2 protein present in the sample. As will be obvious to those of skill in the art upon this disclosure, other reporter antibodies and means for detecting these antibodies can also be used. Quantitative results typically are obtained by reference to a standard curve.

A competition assay can also be used wherein antibodies specific to PLA_2 are attached to a solid support and labeled PLA_2 and sample obtained from the patient having cancer are passed over the solid support. The amount of label detected attached to the solid support can then be correlated to a quantity of PLA_2 in the sample.

Nucleic acid methods can also be used to detect PLA_2 mRNA in a bodily fluid obtained from a patient having cancer. Examples of nucleic acid methods include, but are not limited to polymerase chain reaction (PCR), ligase chain reaction (LCR) and nucleic acid based amplification (NASABA). Reverse transcriptase PCR (RT-PCR) also provides a powerful tool useful in the detection of the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species.

Of the proteomic approaches, 2D electrophoresis is a technique well known to those in the art. Isolation of individual proteins from a sample such as serum is accomplished using sequential separation of proteins by different characteristics usually on polyacrylamide gels.

First, proteins are separated by size using an electric current. The current acts uniformly on all proteins, so smaller proteins move farther on the gel than larger proteins. The second dimension applies a current perpendicular to the first and separates proteins not on the basis of size but on the specific electric charge carried by each protein. Since no two proteins with different sequences are identical on the basis of both size and charge, the result of a 2D separation is a square gel in which each protein occupies a unique spot. Analysis of the spots with chemical or antibody probes, or subsequent protein microsequencing can reveal the relative abundance of a given protein and the identity of the proteins in the sample.

Additional methods obvious to those of skill in the art upon this disclosure for determining PLA_2 levels in bodily fluids can also be used.

Without limiting the instant invention, typically, for a quantitative diagnostic enzyme immunoassay, a positive result indicating that the cancer in the patient being tested or monitored has metastasized is one in which bodily fluid levels of the cancer marker, PLA_2, are elevated above an established enzyme immunoassay (EIA) cut-off of 4.5 ng/ml. However, as will be obvious to those of skill in the art upon this disclosure, alternative EIA cutoffs may be established depending upon the acceptable number of false positive or false negative results for a particular patient group. In addition, in another embodiment, elevated levels of PLA_2 can be determined by comparing measured amounts in the sample of bodily fluid obtained from the human patient having cancer with amounts of this marker in the same bodily fluid type of normal human controls or previously measured amounts in the same patient. That is, if PLA_2 in serum is being measured in the patient, this amount is compared with the amount of PLA_2 in serum of normal human controls or the amount of PLA_2 measured in serum of the same patient previously. By "normal

human control" it is meant a random grouping of males who have not been diagnosed with prostate cancer or any other type of cancer. An increase or elevation in PLA_2 in the patient versus the amount in normal human controls or the amount previously measured in the same patient is associated with a cancer which has metastasized.

Table 1 shows the percentage of individuals identified as positive based upon measurement of PLA_2 in serum by an ELISA with an EIA cut-off of 4.5 ng/ml in normal human controls, patients having benign prostatic hyperplasia, patients having prostatitis, patients having untreated prostate cancer in Stage A, patients having untreated prostate cancer in Stage B, patients having untreated prostate cancer in Stage C, and patient newly diagnosed and treated for progressive metastasis from prostate cancer in Stage D.

Table 1

Serum Levels of PSA and PLA_2

Groups	N	PLA_2 Positive (%) (cutoff 4.5)	PSA Positive (%)
Random Males	1573	3.4	0.0
BPH	70	2.9	27.1
Prostatitis	20	15.0	30.0
Prostate Cancer Stage A	14	7.1	78.6
Prostate Cancer Stage B	41	7.3	87.8
Prostate Cancer Stage C	17	11.8	88.2
Prostate Cancer Stage D	58	81.0	87.9

The PSA Positive value for Random Males is based upon random PSA testing. As demonstrated in this table, the percent of

individuals positive for PLA_2 increases significantly in patients with Stage D prostate cancer as compared to either normal human controls or patients with prostate cancer in Stage A, B or C. Accordingly, measurement of PLA_2 provides a useful means for diagnosing metastatic cancer and/or monitoring the onset of metastasis in patients with prostate cancer which has not yet metastasized.

Table 2 shows the percentage of individuals identified as positive based upon measurement of PLA_2 in serum by an ELISA with an EIA cut-off of 4.5 ng/ml in patients having breast, colorectal, ovarian or testicular cancer classified as either localized, progressive metastatic, metastatic in remission or metastatic stable.

Table 2

Serum Levels of PLA_2

Cancer Type	Localized		Progressive Met		Met Remission		Met Stable	
	N	% Pos	N	% Pos	N	% Pos	N	% Pos
Breast	4	25.0	24	83.3	8	75.0	9	44.4
Colorectal	3	33.3	20	75.0	7	85.7	5	80.0
Ovarian	1	0.0	36	72.2	1	0.0	3	33.3
Testicular	6	50.0	14	64.3	14	28.6	7	28.6

As demonstrated in this table, the percent of individuals positive for PLA_2 also increases significantly in patients with progressive breast, colorectal, ovarian and testicular metastatic cancers as compared to patients with localized cancers. Accordingly, measurement of PLA_2 provides a useful means for monitoring the onset of metastasis in patients with various types of cancer, including prostate, breast, colorectal, ovarian and testicular cancer. Further, measurement of serum PLA_2 levels at the time of diagnosis of

prostate, breast, colorectal, ovarian or testicular cancer or subsequently thereafter serves as an aide not only in the diagnosis of the cancer but also in determining if the newly diagnosed cancer is metastatic.

Further, as shown in Table 2, the percent of individuals positive for PLA_2 decreases in patients with breast, ovarian or testicular metastatic cancer that is in remission or stabilized, from the elevated percent of individuals positive for PLA_2 with progressive metastasis. Accordingly, measurement of PLA_2 provides a useful means to monitor progression, remission, response to therapy or stabilization of various cancers over time. Levels of PLA_2 can be determined in biological fluid samples obtained from the patient at selected times. Times for determining PLA_2 levels for monitoring the progression, remission, response to therapy or stabilization of cancer in a patient can be routinely selected by one of skill in the art in accordance with the patient's history and the type of cancer which is being monitored. An increase in the measured levels of PLA_2 in a patient over time is indicative of progressive cancer. A decrease in the measured levels of PLA_2 in a patient over time is indicative of remission or response to therapy of the cancer. No change in the measured levels of PLA_2 in a patient over time is indicative of stabilization of the cancer.

As also demonstrated in Table 2, elevated PLA_2 levels in a bodily fluid obtained from a patient are also indicative of breast, colorectal, ovarian or testicular cancer in the patient. The percent of individuals positive for PLA_2 with breast, colorectal, ovarian or testicular cancer that is localized, progressive metastatic, metastatic in remission, or metastatic stable is generally greater than random healthy males or females.

Patients diagnosed early with cancer generally have a much greater five-year survival rate as compared to the survival rate for patients diagnosed with distant metastasized

cancer. Thus, diagnostic methods which are more sensitive and early detection of cancers are clearly needed. Accordingly, the present invention also relates to methods for diagnosing cancers, and in particular ovarian cancer or testicular cancer, in a patient by measuring PLA_2 in bodily fluids of the patient suspected of suffering from cancer. In these methods, levels of PLA_2 at least two standard deviations above levels of PLA_2 determined in healthy males are indicative of ovarian or testicular cancer. Levels of PLA_2 in patients suspected of suffering from ovarian or testicular cancer are determined in a biological fluid obtained from the patient in accordance with well known assays as described herein.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art based upon this description. It should be understood, however, that the description, while indicating preferred embodiments of the invention, is given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the present disclosure.

The following nonlimiting examples are provided to further illustrate the present invention.

EXAMPLES

Example 1: Quantitative immunoassay for Type II PLA_2

Microtiter plates were prepared for assays by coating a purified monoclonal antibody (SK088-3C6.16.2; Roshak et al., *J. Biol. Chem.*, 1994, 269, 25999-26005) against type II PLA_2 at a concentration of 2.0 µg/ml in 10 mM Tris-HCl buffer at pH 8.0 overnight at 2-8°C. The microtiter wells were then washed four times with 10 mM Tris-HCl at pH 7.5 containing 150 mM NaCl and 0.05% Tween-20 (TBS-T) and blocked with 1.0% BSA (Sigma A-7888) in 50 mM Tris-HCl at pH 8.0 for one hour at 21-25°C (room temperature). The microtiter plates were then

washed four times again with TBS-T before immediate use in the assay.

For the immunocapture assay, 200 µl of 0.5 µg/ml of a second monoclonal antibody (SK097-1E8.5.2; Roshak et al., *J. Biol. Chem.*, 1994, 269, 25999-26005) against type II PLA_2 conjugated to biotin (Hinatowich et al., *J. Nucl. Med.*, 1987, 28, 1294-1302) in sample diluent (SPD) buffer (50 mM Tris-HCl at pH 8.0, 300 mM NaCl, 0.1% BSA, 0.05% Tween-20, 1% mouse serum (Jackson ImmunoResearch Labs 015-000-120), and 0.02% NaN_3) was added to each well. Also added to the microtiter wells at this step was 10 µl of either type II PLA_2 standard (Levin et al. *Protein Expr. Purif.* 1992, 3, 27-35) in fetal calf serum (HyClone A-1111-L) or patient serum. The plates were then incubated for 1 hour at 21-25°C with shaking. After the incubation, the mixture was removed, the microtiter plates were washed 4 times with TBS-T and 100 µl of avidin-alkaline phosphatase (Sigma A-2527) at 1.0 µg/ml in SPD buffer were added. After a one hour incubation at 21-25°C with shaking, the wells were washed again four times with TBS-T. Finally 200 µl of p-nitrophenyl phosphate at 1.0 mg/ml in 10 mM diethanolamine at pH 9.7 were added to the wells and incubated for 30 minutes at 21-25°C with shaking. Assay absorbance at 405 nm was read for each microtiter well. Assay absorbance values for purified type II PLA_2 standards were used to generate a standard curve, and absorbance values for patient sample assays were compared to the standard curve to determine type II PLA_2 concentrations.

Example 2: Quantitative Assay of Prostate Specific Antigen (PSA) in Serum

PSA levels in serum samples of the same patients were quantified using the TANDEM-E PSA ImmunoEnzyMetric Assay (Hybritech, Inc. Sand Diego, CA) in accordance with the manufacturer's instructions.

What is claimed is:

1. A method of monitoring a cancer in a patient which has not metastasized for the onset of metastasis comprising:

(a) identifying a patient having a cancer that is not known to have metastasized;

(b) periodically analyzing a sample of bodily fluid from the patient for PLA_2; and

(c) detecting levels of PLA_2 in the sample of bodily fluid obtained from the patient, wherein elevated levels of PLA_2 in a sample of bodily fluid are indicative of a cancer which has metastasized.

2. The method of claim 1 wherein the bodily fluid is serum.

3. The method of claim 1 wherein the analysis of the bodily fluid for PLA_2 is by ELISA.

4. The method of claim 3 wherein the established enzyme immunoassay cutoff is 4.5 ng/ml.

5. The method of claim 1 wherein the cancer is breast, colorectal, ovarian, prostate or testicular cancer.

6. A method of diagnosing a metastatic cancer in a patient comprising:

(a) obtaining a sample of biological fluid from a patient; and

(b) detecting the level of PLA_2 in a sample of biological fluid, wherein elevated levels of PLA_2 in the sample are indicative of a metastatic cancer.

7. The method of claim 6 wherein the bodily fluid is serum.

8. The method of claim 6 wherein the analysis of the bodily fluid for PLA_2 is by ELISA.

9. The method of claim 8 wherein the established enzyme immunoassay cutoff is 4.5 ng/ml.

10. A method of diagnosing ovarian or testicular cancer in a patient comprising:

(a) obtaining a sample of biological fluid from a patient; and

(b) detecting levels of PLA_2 in the sample; wherein elevated levels of PLA_2 in the sample are indicative of cancer.

11. The method of claim 10 wherein the biological fluid is serum.

12. The method of claim 10 wherein PLA_2 is detected by ELISA.

13. A method of monitoring progression, remission, response to therapy and stabilization of prostate, breast, ovarian or testicular cancer in a patient comprising measuring PLA_2 levels in biological fluid samples obtained from a patient at selected times wherein an increase in the measured levels of PLA_2 over time is indicative of progressive cancer, a decrease in the measured levels of PLA_2 over time is indicative of remission or response to therapy of the cancer, and no change in the measured levels of PLA_2 over time is indicative of stabilization of the cancer.

14. The method of claim 13 wherein the bodily fluid is serum.

15. The method of claim 13 wherein PLA_2 is measured by ELISA.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/03170

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 33/53, 53/567; C07K 16/00
US CL :435/7.2, 7.21, 7.23, 7.91, 7.92; 436 546; 530/387.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/7.2, 7.21, 7.23, 7.91, 7.92; 436 546; 530/387.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, medline,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/05349 A1 (SMITHKLINE BEECHAM CORPORATION) 12 February 1998, see especially p.1, lines 10-11, p.2, lines 18-22 and Example 2.	13-15
X	YAMASHITA, S. et al. Elevation of Serum Group II Phospholipase A2 Levels in Patients with Advanced Cancer. Clinica Chimica Acta. 1994, Vol. 228, pages 91-99, see entire document.	13-15
X - Y	ABE, T. et al. Group II Phospholipase A2 is Increased in Peritoneal and Pleural Effusions in Patients with Various Types of Cancer. Int. J. Cancer (Pred. Oncol.) 1997, Vol. 74, pages 245-250, see entire document.	13 --- 15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/03170

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	US 5,747,264 A (SCHMIDT, C.J. et al) 05 May 1998, see entire docuemnt.	13-15
A	YAMASHITA, S. et al. Overexpression of Group II Phospholipase A2 in Human Breast Cancer Tissues is Closely Associated With Their Malignant Potency. Cancer. 1994, Vol. 69, pages 1166-1170, see entire document.	1-15